Laboratory Studies with *Leucoagaricus* and Attine Ants 9

P.J. Fisher[1] and D.J. Stradling[2]

[1]*School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry 1st Street, Portsmouth PO1 2DY, UK;* [2]*Hatherly Laboratories, Department of Biological Sciences, University of Exeter, Exeter EX4 4PS, UK*

Formation of *Leucoagaricus* Basidiomes in a Live Nest of *Atta cephalotes*

Möller (1893) was the first to provide detailed descriptions of fungi isolated from ant fungus gardens. In his study the fungi were isolated from the nests of various attine species, including members of the genera *Acromyrmex*, *Apterostigma* and *Cyphomyrmex*, and he described and drew the development of agaricaceous basidiomes obtained from the surfaces of leaf-cutting ants' nests in the field. Accurate diagrams of gongylidia were made. These are the typical swollen hyphal tips of the vegetative mycelium in ants' nests, which developed after inoculation of nutrient agar with fragments of basidiome removed from inner non-fertile tissues. He also successfully grew vegetative mycelium from basidiospores which later formed gongylidia. Möller named the species *Rozites gongylophorus* A. Möller. Weber (1957, 1966), using the fungi isolated from nests of the primitive attines *Cyphomermex costatus* Mann and *Myrmicocrypta buenzli* Borgmeier, and Hervey *et al.* (1977), using the fungus from the ant species *Apterostigma auriculatum* Wheeler, obtained basidiomes in culture, but in no case did they report gongylidia-bearing mycelium. According to Heim (1957) and Singer (1975) both Weber's and Hervey's agarics were specifically identical and congeneric with *Leucoagaricus* and should be known as *Leucoagaricus gongylophorus* (A. Möller) Singer. The combination *L. gongylophorus* was formally proposed by Singer (1986). More recently Muchovej *et al.* (1991) have described *Leucoagaricus weberi* J. Muchovej, Della

© CAB *International* 2002. *Tropical Mycology*, Vol. 1, *Macromycetes* (eds R. Watling, J.C. Frankland, A.M. Ainsworth, S. Isaac and C.H. Robinson)

Lucia and R. Muchovej as a new species and the first agaric found growing in a living nest of *Atta sexdens* L.

This chapter describes the successive formation of four separate basidiome aggregates over a period of 9 weeks in a living nest of the tropical lowland leaf-cutter ant *Atta cephalotes* L.

Colonies of *A. cephalotes* with queens were collected in Trinidad during the months of July and August in 1980, 1986 and 1991 and subsequently maintained in the Department of Biological Sciences at the University of Exeter, UK. They were about 1 year old, with a single fungus garden *c.* 10 cm diameter. In addition to the above, five young *A. cephalotes* colonies were collected by R. Jackman at Gilpin Trace, Roxborough, Tobago, during November 1989 and delivered to the laboratory at Exeter 3 days later, but the fungus gardens were destroyed in transit. To regenerate the colonies, each surviving queen with her workers and brood were placed on a moist sponge cloth in a plastic container and supplied with approximately 20 g of mature fungus garden taken from a laboratory colony of *A. cephalotes* at Exeter, collected in July 1986 in the Caura Valley, Trinidad.

All five of these colonies survived, successfully adopting the new fungus gardens. Cultures were kept at 25–26°C and relative humidity 70–80% in a 12:12 photoperiod. The nests were housed under Perspex containers, isolated on separate boards supported by legs, resting in paraffin moats to prevent ant escape. Each colony was provided with a dish of water and a daily supply of a variety of plant material, principally privet leaves (*Ligustrum ovalifolium* Hassk.), grapefruit skins (*Citrus paradisi* MacFad.), orange skins (*Citrus aurantium* L.) and cultivated rose leaves and petals (*Rosa* sp. hybrid tea, see Fig. 9.1). Expansion of the colonies was normally restricted to 15 cm × 15 cm × 20 cm by their covers, but one colony was eventually rehoused in a larger container 20 cm × 20 cm × 30 cm where the ants ultimately expanded the fungus garden to occupy 75% of the available space with an estimated fresh weight of 1.75 kg.

Fig. 9.1. Ants carrying rose petals to their nest.

In June 1993 an irregular structure with the appearance of an incipient basidiome began to develop in this enlarged colony (Fig. 9.2). Other subsequent aggregations of new basidiomes developed at 2–3 week intervals, usually just above the central line of the fungus garden until after about 10 weeks the process terminated (Fig. 9.3). The addition of inert markers indicated that the turnover time for plant material was about 5–7 weeks, so the basidiomes gradually moved downwards during their formation as new fungus garden was added at the top of the colony and old garden was removed from below by the ants. The ants appeared to feed their larvae preferentially on developing basidiome tissue, perhaps because the fungus was present there in a concentrated form, similar to that of the gongylidia in the fungus garden, which are the principal larval food.

The basidiomes were hollowed out by the ants from the inside (Fig. 9.4), with the result that most of the hymenial tissues were destroyed before they could ripen to produce basidiospores. Some basidiome aggregates were therefore removed at an appropriate stage. Samples of teased tissue taken from the inside of the stipe showed a structure traversed by gloeoplerous hyphae with oleaginous contents which stained deeply with lactofuchsin (Fig. 9.5). Electron micrographs (longitudinal sections) revealed dolipore septa similar to those in the mycelium found in the fungus garden, which furnished additional proof that the structures were basidiomes. One basidiome was sectioned (Fig. 9.6). Squash preparations of hymenial tissue yielded four-spored basidia, 22–32 × 8.5–

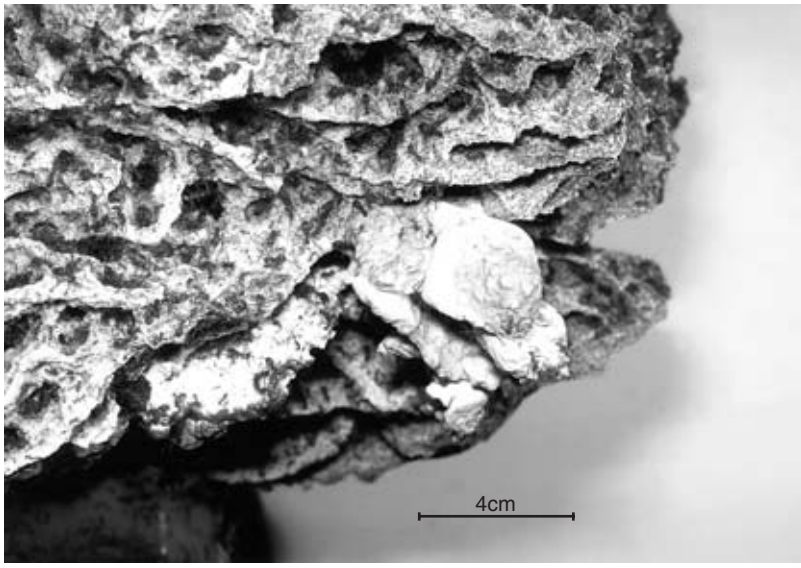

Fig. 9.2. Developing basidiomes of *Leucoagaricus gongylophorus* on a fungus garden in a live nest of *Atta cephalotes*. Scale bar = 4 cm.

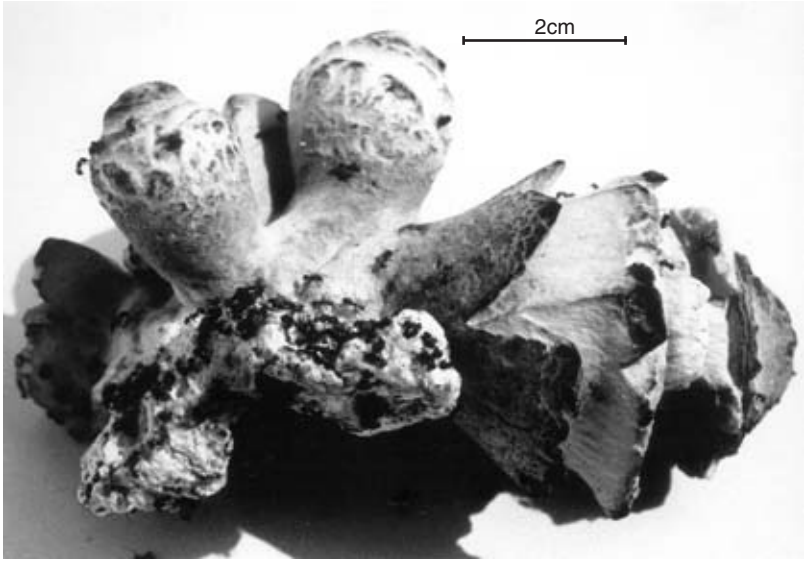

Fig. 9.3. Some stages of development of basidiomes removed from a nest of *Atta cephalotes*. Scale bar = 2 cm.

14 μm and individual basidiospores, 5.7–7.7 × 4.5–5 μm; cheilocystidia, 24–44 × 5.5–8.5 μm, taken from a gill edge, were also present. The strongly dextrinoid spore wall, the lack of apical differentiation into a germ pore or similar structure and the absence of clamp connections are all features typical of *Leucoagaricus*.

Attempts to germinate immature basidiospores failed and although a number of basidiomes were placed on moist cotton wool in a damp chamber, the basidiomes failed to expand to yield mature basidiospores from which gongylidia-forming mycelium could be cultured. To relate the basidiome tissue to the mycelial form of the fungus, ten aggregates of cheilocystidial elements, each aggregate measuring ≤ 1 mm, were then dissected from the hymenium under sterile conditions and plated on to potato dextrose agar (PDA) medium. Thirty fragments of stipe tissue were similarly dissected and incubated on PDA at 25°C. After about 1 week all the incubated fragments showed new mycelial growth and approximately 15 days later typical aggregates of gongylidia (Fig. 9.7) formed at the edges of these colonies. All colonies obtained from fragments of the basidiomes looked and behaved identically to those obtained from the fungus garden.

It is also noteworthy that ants secrete antibiotic substances which suppress the growth of many microorganisms (Maschwitz *et al.*, 1970). One such substance, myrmicacin, $C_{10}H_{20}O_3$ (Schildknecht and Koob, 1971), inhibits the growth of a large number of fungi, including typical soil organisms such as *Penicillium*, and plant epiphytes such as *Cladosporium* and *Alternaria*. This

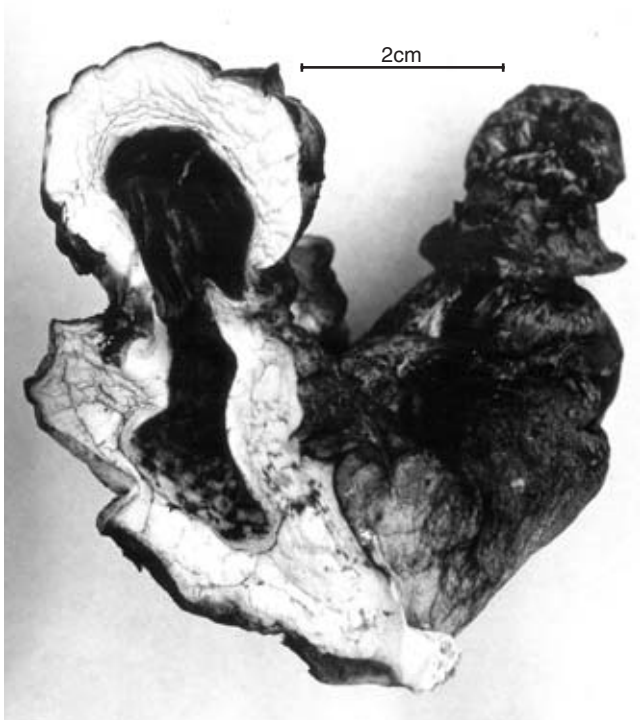

Fig. 9.4. A basidiome which has been hollowed out by *Atta cephalotes*. Scale bar = 2 cm.

indicates that the total mass of basidiome tissue (± 200 g) must have grown from the cultivated fungus since an invading fungus was likely to have been suppressed.

This work therefore strongly suggests a connection between the basidiomes and the mycelium of the fungus garden, although it would have been preferable to obtain further proof from germinating basidiospores. According to Muchovej *et al.* (1991), their description of *L. weberi* in a nest of *A. sexdens* was the first record of any agaricoid fungus in an active nest in which ants were still cultivating the fungus garden. The work at Exeter described a second such event but in a nest of *A. cephalotes*.

The authors' successful method for isolating mycelium from the basidiome tissue to obtain gongylidia reflects Möller's work, but his original herbarium material appears to have been lost. It is regrettable that, except for Möller, none of the other workers reported gongylidia formed by the vegetative mycelium obtained from basidiomes, a feature common to all fungi isolated from true attine fungus gardens. What induces the formation of teleomorphs in a fungus garden remains unknown. In the absence of clamp connections on the mycelium bearing the

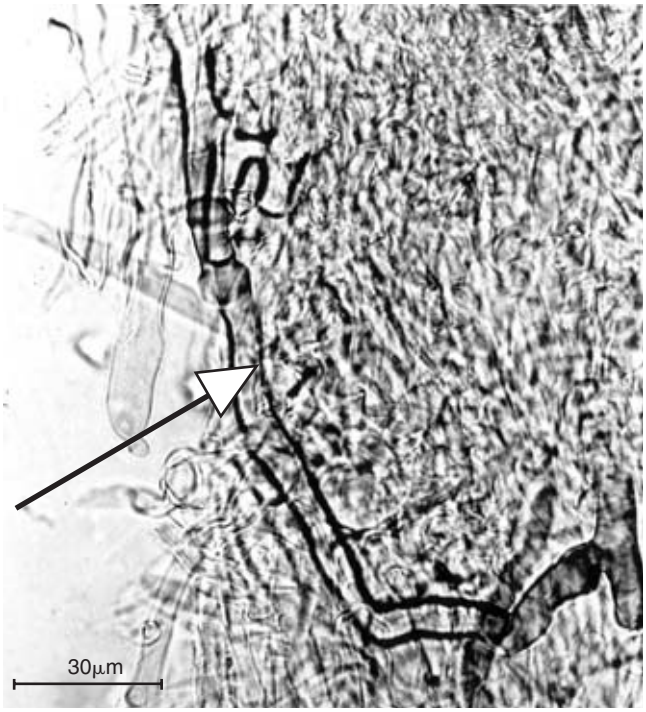

Fig. 9.5. Teased tissue taken from the inside of a stipe showing gloeoplerous hyphae (arrowed). Scale bar = 30 μm.

gongylidia, and observations on the cytological state of the mycelium, it is not known whether the mycelium in the nest is normally monokaryotic or dikaryotic. However, it seems unlikely that after nests had been kept under identical conditions at the Exeter laboratory for many years without apparently forming the teleomorph, sudden cytological changes in the mycelium in the nest should result in formation of basidiomes. It is more likely that some subtle change in the state of the nest had occurred, such as a failure of the ants to remove teleomorph initials for feeding their larvae, thus allowing the basidiomes to grow into more mature structures. This was supported by the fact that the queens of such nests, and consequently the whole colony, went into decline and in some cases died some weeks after the last basidiome had formed. Thus the colonies had probably entered the final ageing process when the ants allowed the basidiomes to form. Communications within an ant's nest must depend heavily on complex chemically induced, feedback mechanisms. Thus, when the queen stops laying eggs the absence of young larvae and the declining need to supply fungus food are probably communicated to the workers and directly affects their fungus-collecting behaviour. Since basidiome tissue is tough and probably more difficult to collect than gongylidia, the former is more likely to be neglected as the need for larval food declines.

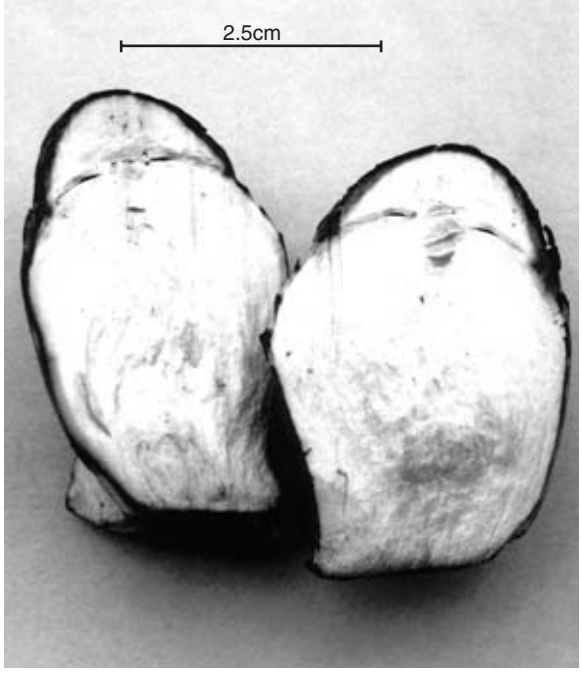

Fig. 9.6. Freshly sectioned developing basidiome. Scale bar = 2.5 cm.

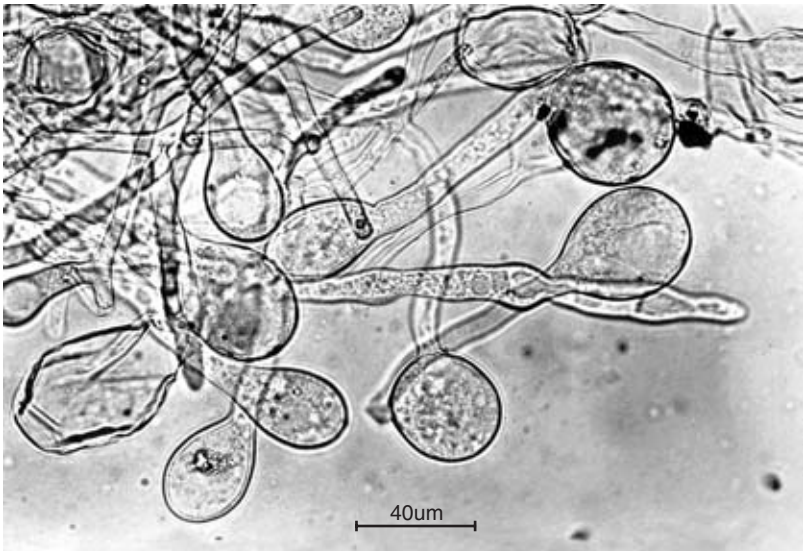

Fig. 9.7. Club-shaped gongylidia, typical of attine fungus gardens and the principal food of ant larvae. Scale bar = 40 μm.

An Assessment of Microfungi in the Fungus Gardens of *Atta cephalotes*

The ant fungus garden is traditionally considered to be a fungal monoculture on a vegetable substratum derived from a wide variety of plant species. The ants actively inoculate the substratum and maintain the culture by various physical and chemical means (Maschwitz *et al.*, 1970; Schildknecht and Koob, 1971). Since Belt (1874) realized that attine ants cultivated a fungus garden for food, the identity of the monoculture has been controversial and a number of names have been suggested (Kreisel, 1972; Hervey *et al.*, 1977; Kermarrec *et al.*, 1986). However, the fungus *L. gongylophorus* (A. Möller) Singer described by Möller (1893) has recently been confirmed as the same by Fisher *et al.* (1994b), although there may be species variations (Muchovej *et al.*,1991). The leaf material imported into the ants' nests by the ant hosts is variable and depends on the plant species available and suitable for the ants in the vicinity of the nest. It is normally cut from living leaves that are still on the host plant. The use of flowers and fruits has also been observed and they have formed useful additions in laboratory nests.

It is well established that plants growing in the wild are covered by an extensive epiphytic fungal community (Dickinson, 1986), and wild plants also contain fungal endophytes (Petrini, 1986). A large variety of fungal species is therefore imported into the nest on a daily basis. In a healthy nest, growth of these species is suppressed by a chemical cocktail which includes myrmicacin and phenylacetic acid. Möller (1893) appears to have been the first person to describe fungi other than *L. gongylophorus* in ant fungus gardens. He mistakenly associated a conidial state of an unnamed hyphomycete with the ant fungus *L. gongylophorus* but nevertheless accurately drew the conidial state of *Escovopsis weberi* Muchovej, Della and Lucia (syn. *Phialocladus zsoltii* Kreisel), a contaminant reported from the fungus gardens of *A. cephalotes* (Möller, 1893; Carmichael *et al.*, 1980; Powell, 1984). Although numerous workers have periodically reported filamentous fungi and yeasts in attine ant nests (Goetsch and Stoppel, 1940; Stahel and Geijskes, 1941; Weber, 1957; Craven *et al.*, 1970; Sihanonth *et al.*, 1973; Kermarrec *et al.*, 1986), there appears to have been no systematic study to evaluate the species and numbers that may be involved.

The research described here was designed to record filamentous fungi and yeasts in three nests of *A. cephalotes* and to examine whether the fungi in the nests corresponded with the epiphytic and endophytic mycota of the food plants. Further, it was investigated whether there might be fungi other than *L. gongylophorus* that had adapted to the environment of the nests and taken up permanent residence there, and whether environmental pressure on the colony such as an enforced diet might have adversely affected the ability of the ants to maintain a monoculture.

The fungus gardens of three colonies of *A. cephalotes* were sampled. Two of these (Q1 and Q2), collected from the field as 1-year-old colonies in the Arima

Valley, Trinidad, West Indies in 1991, were subsequently maintained in the laboratory at Exeter at 25°C, in a 12:12 h light regime and relative humidity > 70%. At the time of sampling, both colonies comprised two fungus gardens of approximately 2 l each. Prior to sampling they had been subjected to a strict 3 months regime of the same *Quercus ilex* L. (holm oak) leaf material collected on the Exeter University campus to test whether the mycota of the leaves could be detected in the nests. Colony Q1 was subsequently supplied with leaves of *Rosa* sp. (hybrid tea) for a further 3 months (Q1R) and resampled for similar tests. The third colony (FT), which was much larger than the other two, was sampled in its natural environment, located in steeply sloping terrain among clumps of bamboo at Noel Trace, St Augustine, Trinidad. The principal foraging trail for the ants, at the time of sampling, extended for 100–200 m to a cultivated garden where leaf pieces were cut from several trees including *Persea americana* Mill. and *Codiaeum variegatum* (L.) Blume. Samples of fungus garden were obtained from a nest chamber exposed by digging, and of waste material by taking it from ants emerging from a number of nest entrances at the base of the nest. The fungus gardens of all the nests were sampled by dividing the nest approximately into an upper and lower region. Then 30 pieces of fungus garden (approximately 0.5 cm^3) consisting of many individual leaf units were sampled from each region and placed immediately into individual sterile glass tubes. Each of these 30 pieces was subsequently dissected into the separate leaf units which rarely measured more than 2 mm^3. These represented fragments of leaf material cut by the ants during the preparation of the fungus garden (Fig. 9.8). Möller (1893) also recorded this fine division of the substratum by the ants. Units of this divided material were taken at random and plated on 1.5% malt extract agar medium (Fig. 9.8) supplemented with 250 mg l^{-1} Terramycin (MEA + T) to inhibit bacterial growth. One hundred pieces each from the upper and lower nest regions were plated. In addition, 100 units of discarded waste from each nest were treated similarly. The process was repeated for nest FT, except that 4 days elapsed between collection and leaf particle plating. Leaf units were incubated for up to 6 weeks at 20 ± 2°C depending on the growth rates of the fungi that emerged (Fig. 9.9). Fungi other than the ant food fungus, *L. gongylophorus*, were isolated by transferring hyphal tips to 2% MEA plates. Near-UV light (Philips TL 40W/05) was used for up to 3 weeks to induce sporulation.

Identification was normally possible after sporulation. *L. gongylophorus*, which has no known anamorph, could be readily identified on the original culture plates by its typical white colonies and bunches of gongylidia (Fig. 9.9). Leaves of *Q. ilex* fed to colonies Q1 and Q2 only, leaves of *Rosa* sp. fed to colony Q1 only, and leaves of *P. americana* and *C. variegatum* collected from the trees being used by the ants as food plants in Trinidad were screened separately for a qualitative presence of epiphytic and endophytic fungi. For the former, three leaves of each species were agitated in 100 ml of sterile water for 1 h, and 0.1 ml aliquots were pipetted on to three Petri dishes containing solid medium 1.5% MEA + T. For the endophytes, leaves were surface sterilized using sodium

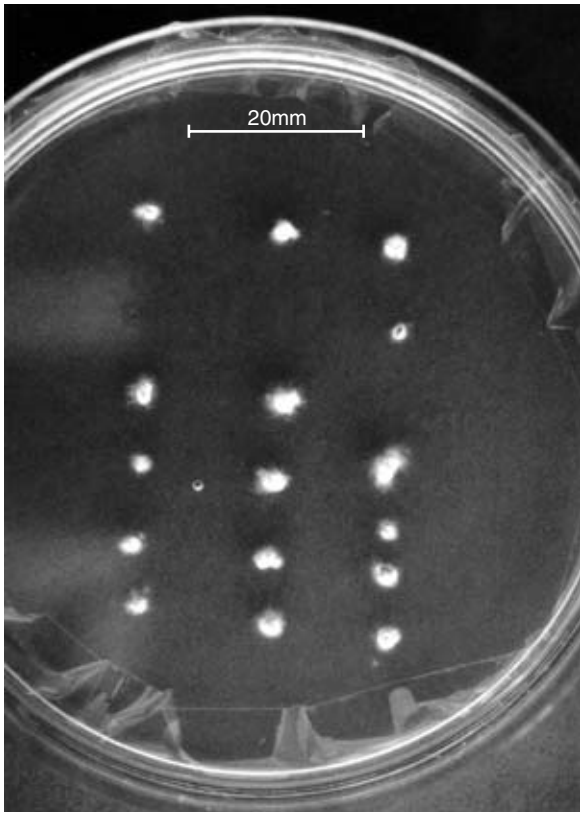

Fig. 9.8. Leaf fragments cut by the ants plated on nutrient agar growing only *L. gongylophorus* after 4 weeks. Scale bar = 20 mm.

hypochlorite and ethanol as described by Fisher *et al.* (1994a) . An estimate of the fungi that might be carried into the nest on the bodies of the ants was made by allowing ten of the leaf-bearing foragers to traverse ten separate 1.5% MEA + T plates for 2 h and then incubating the plates at 20°C for 5 days. The relative abundance of filamentous microfungi in the upper and lower regions of the fungus gardens from the three colonies sampled were compared using χ^2 analysis.

When considering the results of this survey, it is important to recognize that the attine fungus garden provides the principal larval food source in the form of gongylidia, and more rarely basidiome tissue of *L. gongylophorus*. The attine nests do not appear to be axenic cultures of *L. gongylophorus* (Table 9.1) if it is assumed that at least some of the fungi isolated from the nests were present in the nests in a mycelial form and not only as ungerminated conidia. When individual leaf fragments that made up the fungus garden were plated on to MEA + T (Fig. 9.8), a substantial proportion (21–53%) gave rise to cultures of only *L. gongylophorus* (Table 9.1). Many of these came from the upper section of the fun-

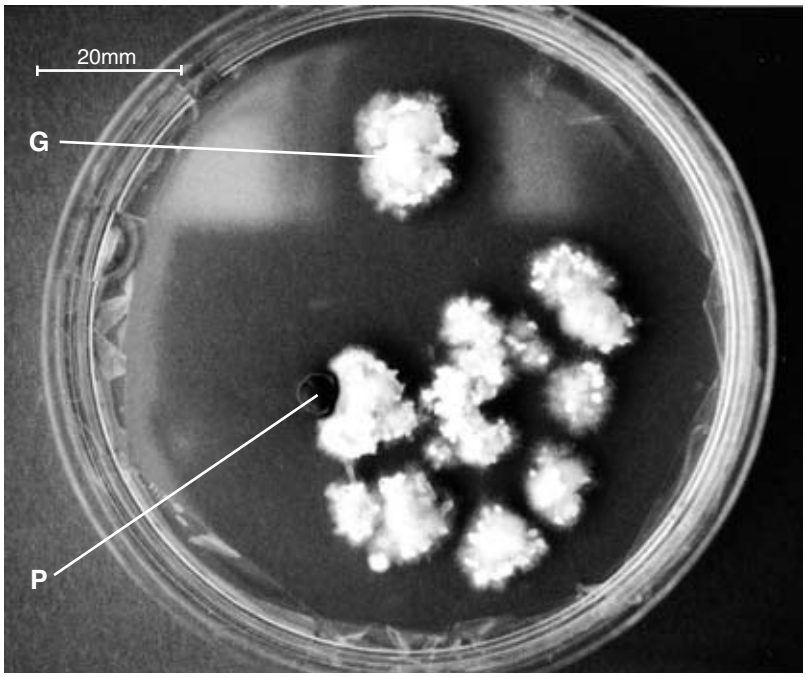

Fig. 9.9. Eight-week-old cultures of *L. gongylophorus* growing from leaf fragments showing clumps of hyaline gongylidia formations (arrowed G). The arrowed colony (P) is *Phomopsis quercella*, an endophyte from the leaves of *Q. ilex*. Scale bar = 20 mm.

gus gardens (Table 9.1), which was remarkable considering that these may have been placed in position by the ants only 24 h previously.

Möller (1893) also commented on rapid colonization of leaf fragments by the basidiomycete in field colonies. The highest colonization frequency of *L. gongylophorus* was recorded from nest Q1R (94%), the lowest from the field nest FT (72%). The latter may be partly due to the 4 day delay between sampling in Trinidad and particle isolation in the laboratory at Exeter. This would have led to some deterioration of the *L. gongyloporus* culture because of the overgrowth of other fungi in the absence of ant husbandry. That isolated pieces of fungus garden are subject to rapid deterioration was recorded previously by Fisher *et al.* (1994b), who failed to grow basidiomes of *L. gongylophorus* to maturity after they had been removed from a laboratory nest with large portions of the fungus garden because of the rapid overgrowth of fungal contaminants. This, and experimental evidence that *L. gongylophorus* shows no signs of antagonism against other fungi in laboratory culture (Stradling and Powell, 1986), suggests that, unlike many basidiomycetes, *L. gongylophorus* has a low capacity for allorecognition and is probably entirely dependent on ant husbandry to suppress incoming microfungi. Möller (1893) made similar observations on isolated fungus garden fragments and in abandoned nests.

Table 9.1. Frequency of occurrence of fungal species on 100 leaf fragments from the upper (U) and lower (L) regions of fungus gardens from three colonies of *Atta cephalotes* (FT, field collected (Trinidad); Q1, Q2, laboratory colonies supplied with *Q. ilex* leaves; Q1R, colony Q1 supplied for 3 months with *Q. ilex* and subsequently for 3 months with leaves of *Rosa*).

	FT		Q1		Q1R		Q2		
	U	L	U	L	U	L	U	L	W
Leucoagaricus gongylophorus	63	74	92	68	91	97	87	81	
(A. Möller) Singer	(37)	(42)	(42)	(0)	(56)	(51)	(60)	(19)	
Trichoderma hamatum (Bonorden) Bainier	4	2	0	0	0	0	0	0	+
Cladosporium herbarum (Pers.) Link	0	2	0	1	0	0	0	0	+
Cladosporium oxysporum Berk. & Curtis	1	0	0	1	2	0	0	0	+
Phoma nebulosa (Pers. & Fr.) Berk.	0	2	0	0	0	0	0	0	+
Phyllosticta ghaesembillae Koord.	17	6	0	0	0	0	0	0	
Glomerella cingulata: (Stonem.) Spaulding & V. Schrenk	3	4	0	0	0	0	0	0	
Trichoderma longibrachiatum Rifai	2	0	0	0	0	0	0	0	
Aureobasidium pullulans (de Bary) Arnaud	0	0	1	4	0	1	0	3	+
Fusarium solani Schlecht.	0	0	0	3	0	0	0	0	+
Aspergillus niger v. Tieghem	0	0	0	2	0	0	0	0	+
Epicoccum nigrum Link	0	0	2	4	0	0	2	6	+
Gliomastix murorum (Corda) Hughes	0	0	4	33	0	0	2	16	
Phomopsis ilicina v.d. Aa ined. syn. *Phyllosticta ilicina* Sacc.	0	0	0	3	0	0	0	4	
Phomopsis quercella (Sacc. & Roum.) Died.	0	0	0	4	0	0	3	0	
Phomopsis glandicola (Lév.) Gonz. Frag.	0	0	0	0	0	0	2	0	
Fusarium sp.	0	0	0	0	0	2	0	0	+

Phomopsis culmorum (W.G.Sm.) Sacc.	0	0	0	0	0	0	0	6	+
Yeasts	19	33	38	41	35	42	24	27	+
Sterile mycelia	15	9	10	2	2	4	9	19	+
Sterile leaf fragments	2	0	3	2	5	0	2	1	
No. of species per leaf fragment	1.32	1.32	1.50	1.72	1.35	1.46	1.31	1.63	
No.of species per leaf fragment less yeasts	1.12	0.93	1.12	1.27	0.95	1.04	1.05	1.35	

W, species found in both nest and waste (+).
Figures in parenthesis are leaf fragments occupied by *L. gongylophorus* only.
Fungi found in waste only: Field fungus garden collection (Trinidad): *Aureobasidium pullulans*, *Cylindrocarpon destructans* (Wollenw.) C. Booth, *Fusarium pallidoroseum* (Cooke) Sacc.; *Phialocladus zsoltii* Kreisel; *Phomopsis persea* Zerova. Fungus garden Q1: *Penicillium* sp., *Rhizopus stolonifer* (Ehrenb.) Lind, *Trichoderma harzianum* Rifai. Fungus garden Q1R: *Aspergillus flavus* Link, *Cladosporium herbarum*, *Cladosporium sphaerospermum* Penz., *Syncephalastrum racemosum* Cohn : J. Schröt. Fungus garden Q2: *Trichoderma harzianum*, *Epicoccum nigrum*.

The ant–fungus association is an obligate mutualism and the maintenance of the fungus culture is essential to the survival of the colony. The leaf material, which largely makes up the plant portion of the nests of attine ants, is nutritionally valuable to endophytic and epiphytic fungi. This often variable material serves as the substratum for *L. gongylophorus*, and also renders the nest attractive to a wide range of microfungi, which are introduced with the leaves and on the bodies of the ants as spores and mycelium. Biologically active secretions by the workers' metapleural glands have been shown to suppress the germination of fungal spores (Schildknecht and Koob, 1971) and are one element of nest 'hygiene'. Another is the removal of refuse by workers which collect particles of refuse in the infrabuccal pocket and eject them outside the nest (Bass, 1993).

The identity of microfungi from outside the nest reflects mostly the epiphytic and endophytic fungal community of the plants collected by the ants as a fungal resource. This is suggested by the similarities between the taxa isolated from the colonies of Q1 and Q2, both supplied with only *Q. ilex* leaves, and the differences in taxa composition in Q1R and FT which had different leaf supplies (Tables 9.1 and 9.2). Seventeen taxa, a number of sterile mycelia, and yeasts were isolated from the fungus gardens (Table 9.1). There is no evidence to suggest that any of the filamentous fungi have adapted to become permanent colonizers of the fungus gardens. For example, *Gliomastix murorum* (Corda) Hughes, recorded as an epiphyte from leaf washings of *Q. ilex*, was isolated 37 times from the fungus garden Q1 but was absent in the same nest Q1R after the substratum had been changed to *Rosa* sp. *Cladosporium herbarum* (Pers.) Link and *Cladosporium oxysporum* Berk. & Curtis isolated from nests FT and Q1R (Table 9.1) are known to be cosmopolitan in distribution and occurred as endophytes or epiphytes in the leaves of most of the resource plants (Table 9.2). Both *Phomopsis ilicina* (Aa ined., syn. *Phyllosticta ilicina* Sacc.) and *Phomopsis quercella* (Sacc. & Roum.) Died., present in nests Q1 and Q2 but not Q1R, were also recorded as endophytes in *Q. ilex* leaves (Table 9.2). They have previously been recorded as among the four most frequently occurring endophytes in leaves of *Q. ilex* (Fisher *et al.*, 1994a). These two fungi are usually specific to *Q. ilex* leaves so they were probably imported into the fungus garden with the leaves. Other fungi recorded as epiphytes and endophytes of the food plants and also present in the fungus garden are listed in Table 9.2. Only *E. weberi* (syn. *Phialocladus zsoltii*; Muchovej and Della Lucia, 1990), reported repeatedly from the waste debris of *Atta* nests in the wild (Möller, 1893; Carmichael *et al.*, 1980; Powell, 1984), has also been recorded from waste in this study (Table 9.1). Recently, another species of *Escovopsis*, *E. aspergilloides* Seifert, Samson & Chapela, has been recorded from nests of *Trachymyrmex ruthae* Weber in Trinidad (Seifert *et al.*, 1995).

A comparison between the mycota in nests Q1 and Q1R showed qualitative and quantitative changes in species composition when the substratum of the nest was changed from *Q. ilex* to *Rosa* sp. The former is due to the importation of different fungal species from the rose leaves (Table 9.1). The change in substratum was also accompanied by a significant reduction in the frequency of fil-

Table 9.2. Some of the fungi isolated as endophytes and epiphytes from fresh leaves, used by the ants to supply the fungus gardens, and subsequently isolated from the fungus gardens.

Leaf species	Endophytes	Epiphytes
Quercus ilex	*Phomopsis ilicina*	*Cladosporium herbarum*
	Phomopsis quercella	*Aureobasidium pullulans*
	Aureobasidium pullulans	*Gliomastix murorum*
	Epicoccum nigrum	Yeasts
	Yeasts	
Persea americana	*Phyllosticta ghaesembillae*	*Fusarium solani*
	Phoma nebulosa	*Cladosporium oxysporum*
	Glomerella singulata	*Aureobasidium pullulans*
	Yeasts	Yeasts
Codiaeum variegatum	*Phyllosticta ghaesembillae*	*Cladosporium herbarum*
	Aureobasidium pullulans	*Aureobasidium pullulans*
	Yeasts	Yeasts
Rosa (hybrid tea)	*Aureobasidium pullulans*	*Cladosporium sphaerospermum*
	Cladosporium herbarum	

amentous fungi in both upper and lower regions of the fungus garden (χ^2 = 8.91, $P < 0.01$; $\chi^2 = 54.07$, $P < 0.001$, respectively). This reduction in the total numbers may be due to a smaller number of fungi imported into Q1R with the rose leaves, but it could also reflect the improved 'colony fitness' after the diet change from the dry and tough foliage of *Q. ilex* to the more succulent leaves of *Rosa* sp. 'Colony fitness' is defined here as the productivity of the colony in terms of importation of leaf material and fungus garden construction. In laboratory colonies this switch between food plants was characterized by a marked improvement of 'colony fitness' (D. Stradling, Exeter, 1990, personal communication). In the field, *A. cephalotes* colonies collect material from a wide range of tropical rainforest trees (Cherrett, 1968) and are characterized by frequent switching between plant species, but nothing is known as to how this reflects on 'colony fitness'. Comparisons of the incidence of filamentous microfungal contaminants between the upper and lower portions of each nest and between colonies revealed significant heterogeneity. For example, higher numbers were recorded from the lower portions of nests Q1 and Q2 but the reverse for colony FT (Table 9.1). There are many unknown variables that could account for this anomaly. One possibility is that in the cleaner environment of the laboratory fewer contaminants are present on the material imported to the upper portion of the fungus garden where the ants always first deposit new plant material. Furthermore, leaves from temperate vegetation contain fewer endophytes than those from the tropics (Fisher *et al.*, 1993).

The presence of yeasts was widespread in all the fungus gardens (Table 9.1), an observation in agreement with that made previously by electron microscopy of fungus gardens of *A. cephalotes* by Craven *et al.* (1970). For the four ant

colonies sampled, between 20 and 40% of leaf fragments showed the presence of yeasts, but no attempt was made to distinguish different taxa. Craven *et al.* (1970), using SEM techniques, found that imported leaf material lacked detectable yeast cells. However, the widespread occurrence of yeasts on leaves collected from the field (Table 9.2), and their recovery from nests (Table 9.1) suggests that they are imported into the nest on the leaf substratum.

In separate experiments, when ants were allowed to traverse agar plates for 2 hours, both filamentous fungi and yeast contaminants were evident. *Cladosporium* spp. were present on nine out of ten plates (3.10 ± 0.48 colonies per plate), *Aureobasidium* spp. on six out of ten plates (1.10 ± 0.35 colonies per plate) and numerous yeast colonies on all ten plates. This suggests that most other fungi were not imported into the nests on the ants but on the leaves. It would be reasonable to suppose that fungi such as *Cladosporium* spp. and *Aureobasium* spp., both of which readily produce large numbers of conidia, are brought into the nest as spores. Endophytes such as *P. ilicina* and *P. quercella* may survive the physical and chemical cleaning processes by the ants for varying periods as mycelium within the leaves.

Yeast trails were frequent on the test plates, suggesting that these fungi are frequently carried on the ants and therefore have the potential for widespread dispersal within the fungus garden matrix. This has been separately confirmed by the leaf piece assays (Table 9.1) and by Craven *et al.* (1970).

The use of inert markers, such as spines of the leaves of *Ilex aquifolium* which the ants deposit in the nests, has demonstrated that the ant fungus garden is a dynamic entity where new nest material is added at the top and over a period of 5–7 weeks descends to the base to be discarded eventually by the ants as waste. Thus there is a complete turnover of the fungus garden material within approximately 7 weeks, and the ultimate fate of all the microfungi attached to the substratum is as discarded waste (Table 9.1). This offers an explanation for the earlier mentioned disappearance of *G. murorum*, isolated 37 times from the fungus garden Q1 but lost from the same fungus garden (Q1R) after the substratum was changed.

Of the 300 waste fragments collected from just outside the three original fungus gardens (FT, Q1, Q2), none grew *L. gongylophorus*. It appeared to have been replaced by numerous fungi which for the most part differed from those in the gardens (Table 9.1), a finding in accord with Craven *et al.* (1970). In comparison, leaf fragments of the fungus garden that were actively tended by the ants yielded fewer filamentous contaminants per fragment. However, the total disappearance of *Leucoagaricus* from the waste fragments once again underlines the poor competitive ability of this basidiomycete.

The mycelial dynamics of *L. gongylophorus* in relation to the fungal contaminants which may enter the nest are unknown. It would be desirable to study them, although the difficulties of doing so would be considerable, partly because each contaminant is likely to interact differently with *L. gongylophorus* and also because any disturbance of the nest would affect the ant husbandry.

Acknowledgements

We thank Professor D.N. Pegler for confirming the identification of *L. gongylophorus* and Dr C.R. McDavid for collection and identification of leaf material in Trinidad. The original published source for Figures 9.1–9.8 was *Mycological Research* 98, pp. 884–888, 1994 and 100, pp. 541–546, 1996.

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